Dispatches

Nuclear Membrane: Nuclear Envelope PORosity in Fission Yeast Meiosis

The fission yeast *Schizosaccharomyces pombe* undergoes closed mitosis but 'virtual nuclear envelope breakdown' at anaphase of meiosis II, in which the nuclear envelope is structurally closed but functionally open.

Shelley Sazer

Open any biology book and you will find a section on mitosis that is most likely a description of the open mitosis of animal cells. During interphase in eukaryotes (Figure 1A), the nuclear envelope (NE) sequesters the chromosomes from the cytoplasm but Ran GTPase-dependent transport through the nuclear pore complex (NPC) allows regulated communication between the nucleus and the cytoplasm (reviewed in [1]). The proteins that regulate the nucleotide-bound state of Ran, the nuclear chromatin-bound guanine-nucleotide exchange factor and the soluble cytoplasmic GTPase-activating protein, cause Ran–GTP accumulation in the nucleus. The resulting Ran–GTP gradient across the NE is essential for nucleocytoplasmic transport (reviewed in [1]).

During mitosis in higher eukaryotes (Figure 1B), at nuclear envelope breakdown (NEBD), the gradient across the NE is dissipated and replaced by a chromosome-based Ran–GTP gradient essential for spindle assembly in animal cells (reviewed in [1]). The absence of a NE allows the spindle to contact and separate the chromosomes. It also causes soluble cellular constituents previously enriched in the nucleus or the cytoplasm to become distributed throughout the cell.

Rarely mentioned in textbooks is another mitotic strategy, the closed mitosis of many 'lower' eukaryotes such as yeast and other fungi, during which the NE remains intact (Figure 1F,H). The interaction of the spindle with the chromosomes does not require NEBD because at mitosis the spindle pole bodies (centrosome equivalents) are embedded in the NE and assemble an intranuclear spindle (Figure 1F,H). It is not known whether a mitotic Ran–GTP gradient exists in yeast [2].

Between the extremes of open and closed lies a diverse set of mitotic strategies, some of which were described decades ago, that blur this distinction [3,4]. In semi-open mitosis the poles of the NE open and provide the cytoplasmic spindle microtubules access to the chromosomes (Figure 1C,D). More recently, Steve Osmani's group made the surprising discovery that during what was thought to be classical closed mitosis in the filamentous fungus *Aspergillus nidulans*, the NPC loses some of its components and its ability to maintain the normal permeability barrier between the nucleus and the cytoplasm [5] (Figure 1E,F), raising the question of whether mitosis in which the NE is ultrastructurally intact but functionally porous can be correctly termed open or closed (reviewed in [6,7]).

As reported in this issue of *Current Biology*, Arai *et al.* [8] and Asakawa *et al.* [9], working in *Schizosaccharomyces pombe*, now describe virtual nuclear envelope breakdown (V-NEBD), which further challenges the open/closed dichotomy. Although, ultrastructurally, the NE is

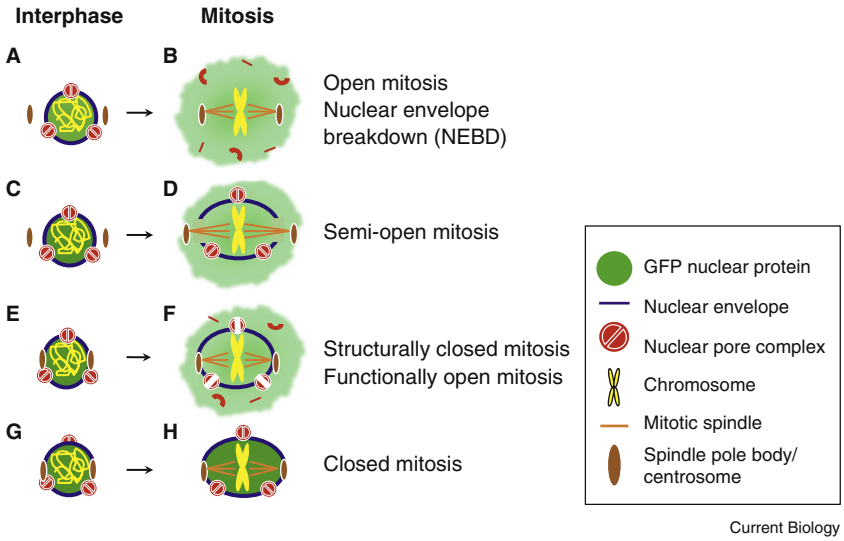

Figure 1. Differences between open, semi-open, structurally closed/functionally open, and closed mitosis.

(A,C,E,G) During interphase in all eukaryotes, the NE physically separates the chromosomes from the cytoplasm. NPCs traverse the envelope and permit passive diffusion of small cargoes and receptor-mediated transport of larger cargoes regulated by the Ran GTPase. (B,D,F,H) During mitosis, the microtubules of the mitotic spindle, organized by the centrosome in higher eukaryotes or the spindle pole body in lower eukaryotes, contact and separate the duplicated chromosomes. Prior to open mitosis (B), the centrosomes are cytoplasmic (A), and NE breakdown (NEBD) results in the mixing of nucleoplasm and cytoplasm. In semi-open mitosis (D), the NE opens at the poles allowing the microtubules to enter the nucleus, and mixing of nucleoplasm and cytoplasm. In closed mitosis (F,H), the NE remains intact, the spindle pole bodies are cytoplasmic in interphase but are embedded in the NE at mitosis where they nucleate formation of an intranuclear spindle. The permeability barrier across the NE is maintained during mitosis in *S. pombe* and other yeasts (H) but in *Aspergillus nidulans* and other filamentous fungi (F) changes in the NPCs allow the equilibration of proteins between the nucleus and the cytoplasm.

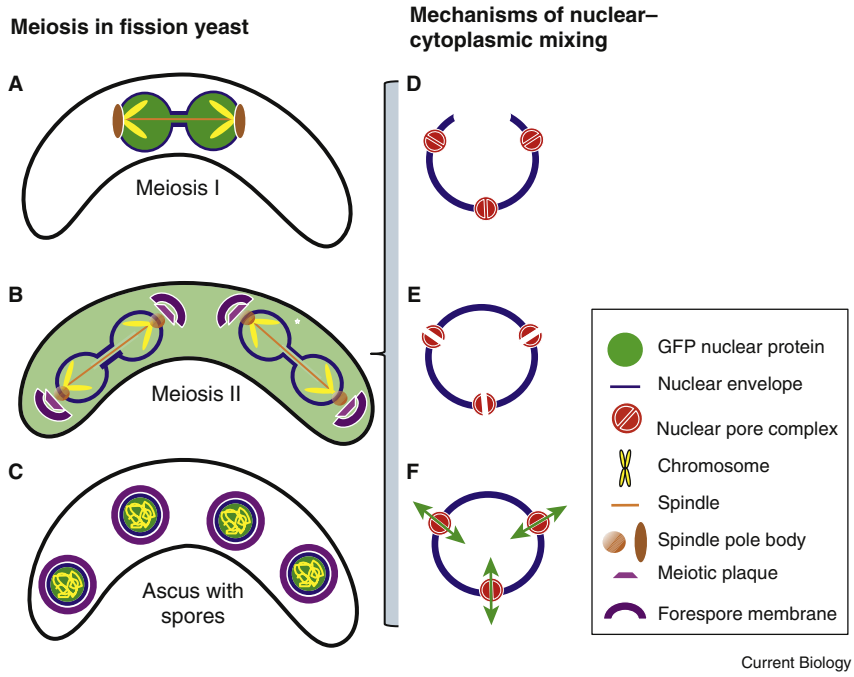

Figure 2. Nuclear proteins relocalize to the cytoplasm during meiosis II in *S. pombe*.

Meiosis is the process that occurs during the sexual stage of development in eukaryotes. This is accomplished in two stages: meiosis I (A) is similar to mitosis, in which DNA replication is followed by chromosome segregation producing two nuclei with the same DNA content of the parent. In meiosis II (B), there is no DNA replication so the products of this 'reductional division' (animal cell gametes or yeast spores) have one-half the DNA content of the parent. In fission yeast, entry into meiosis II triggers formation of a specialized double membrane called the forespore membrane on the cytoplasmic face of the SPB (B) that expands to surround each of the four nuclei (C) [14]. It is between the two membranes that the spore wall is eventually assembled. At anaphase of meiosis II (B) there is a mixing of nucleoplasm and cytoplasm that could be caused by breaks in the NE (D), changes in NPC permeability (E), or changes in the regulation of nucleocytoplasmic transport (F).

intact during mitosis, meiosis I, and meiosis II (Figures 1H and 2A,B), the two groups independently discovered an unexpected transient mixing of nuclear and cytoplasmic components at the initiation of anaphase of meiosis II, accompanied by the nuclear entry of the RanGAP Rna1 and a change in NE permeability.

The two papers [8,9] propose somewhat different but not mutually exclusive models to explain meiosis II-specific V-NEBD. Disruption of the normal barrier between the nucleus and the cytoplasm could be the result of changing the properties of the NE (Figure 2D) or the NPC (Figure 2E), and/or altering nucleocytoplasmic transport (Figure 2F). These possibilities will be addressed here by discussing the new insights provided by the Arai *et al.* [8] and Asakawa *et al.* [9] papers in the context of our current state of knowledge while setting the stage for what will surely be a wave of exciting new studies following up on these reports.

Well-documented changes in spindle pole body structure and protein composition at anaphase of meiosis II [10–13] could disrupt the anchoring of the spindle pole body (SPB) in the nuclear membrane, resulting in a transient hole. These SPB changes occur as the meiotic plaque assembles on the cytoplasmic face of the SPB (Figure 2B), forming a platform for the fusion of endoplasmic reticulum derived vesicles to form the forespore membrane [14]. In fact, V-NEBD and the presence of Rna1 in the nucleus depend on entry into meiosis II, can be prevented by blocking endoplasmic reticulum membrane trafficking or spore formation, and can be accelerated by inducing premature entry into meiosis II [8]. However, because V-NEBD occurs even when SPB changes are prevented by mutation, the critical initiating event may be at the membrane trafficking stage [8].

As suggested by Arai *et al.* [8], it is also possible that, as in the case of the pim1-d1 mutant that carries a temperature-sensitive mutation in the Ran guanine nucleotide exchange factor [15], the endoplasmic reticulum may be unable to provide adequate membrane for both NE growth and forespore membrane assembly at meiosis II, thereby changing the structure and/or permeability of the NE. However, rigorous electron microscopic evidence from Asakawa *et al.* [9] shows that the NE remains intact in cells undergoing V-NEBD. Although it could be argued that there is a hole too small or transient to be seen, the data presented are consistent with the hypothesis that nuclear integrity is maintained during meiosis II.

Similar to the situation in *A. nidulans* mitosis [5], the components of the NPC could change during meiosis II in *S. pombe*, allowing an increase in the functional size of the NPC and equilibration of soluble proteins across the NE. Arai *et al.* [8] and Asakawa *et al.* [9] tested the location of almost every NPC protein and found that all are NE-localized in meiosis II. These studies do not rule out the possibility that reorganization or biochemical modification of proteins within the NPC changes its properties, but from their data the authors conclude that the pores have normal structure and composition.

A Ran–GTP gradient across the NE regulates signal-mediated nucleocytoplasmic transport by influencing the binding between transport cargoes and their carriers (reviewed in [1]). Abnormal introduction of Ran GAP into the nucleus dissipates the gradient and blocks nuclear export and import [16,17]. In *S. pombe*, Rna1 (Ran GAP) is cytoplasmic at steady state but Arai *et al.* [8] and Asakawa *et al.* [9] show that it transiently enters the nucleus at meiosis II, just as it does in *Aspergillus nidulans* mitosis [5]. If, like Rna1 in the budding yeast *S. cerevisiae*, fission yeast Rna1 shuttles between the nucleus and the cytoplasm [18], increased import, decreased export, or both could alter its localization.

One model consistent with these results and proposed by Asakawa *et al.* [9] is that the nuclear entry of Rna1 and the subsequent dissipation of the Ran–GTP gradient is the initiating event for V-NEBD. Artificially forcing Rna1 into the nucleus causes V-NEBD in meiosis II [9] but the biochemical basis

of this equilibration and the answer to the chicken-and-egg question of whether changes in nucleocytoplasmic transport cause nuclear entry of Rna1 or vice versa is unknown.

One possibility raised by Asakawa *et al.* [9] is that the physiological role of V-NEBD is to exclude certain proteins from the nucleus prior to spore formation. Consistent with this possibility, it is known that the volume of spore nuclei is less than that of growing cells [19]. V-NEBD, like NEBD, may also be a means of dissipating the Ran–GTP gradient across the NE and allowing the establishment of a chromosome-based Ran-GTP gradient known to be important for spindle formation in animal cells. However, V-NEBD is first observed at meiotic anaphase II, well after spindle assembly [8].

Deciphering the biological role of V-NEBD will be a challenging task but the fact that it is specific to meiosis II may provide important clues. Meiosis II-specific changes in spindle pole body anchoring in the NE could explain V-NEBD and its cell-cycle stage specificity but would not be consistent with the absence of an ultrastructural defect in the NE [9]. Regardless of the mechanism, it will be important to determine the functional relevance of V-NEBD at meiosis II by accomplishing the difficult task of creating conditions in which V-NEBD is prevented and then monitoring the consequences (see Figure 2C).

Open and closed mitosis are extremes of a process with many variations (Figure 1). The two new papers from Arai *et al.* [8] and Asakawa *et al.* [9] add a fascinating new twist by describing V-NEBD, which is structurally closed but functionally open and occurs only at meiosis II in *S. pombe,* an organism in which mitosis and meiosis I are structurally and functionally closed. The surprising observation of V-NEBD alone raises important questions about how and why it happens and why it happens only at meiosis II. In addition to furthering our understanding of yeast meiosis, the answers to these questions will bear on understanding the fundamental differences between open and closed mitosis and may provide insight into the evolution of nucleated cells.

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Reproductive Strategies: How Big Is Your Love?

In swordtails, gene copy number variation is associated with alternative male mating strategies, size and puberty. Though it is unclear how the different aspects are linked mechanistically, the nature of the gene, a melanocortin receptor, suggests avenues for future inquiry.

Florian Maderspacher

There are many paths to success, a truism illustrated in biology by the diversity of forms, lifestyles, and species. Even within a species, lifestyles can vary, as is exemplified in the alternative strategies males use for accomplishing their one purpose in life, mating. Male side-blotched lizards, for instance, come in three sizes — conveniently colour labelled orange, blue and yellow — that differ in mating behaviour [1]. Similarly, in a marine isopod, there are three types of male that vary considerably in their appearance yet are equally successful at reproducing [2]. Most spectacularly, perhaps, male Australian cuttlefish use their superior morphing skills to disguise temporarily as females and sneak their sperm into a mating couple [3]. Now, as they report in a recent issue of *Current Biology*, Kathrin Lampert, Manfred Schartl and colleagues [4]